Suspected and clinically definite multiple sclerosis: the relationship between CSF immunoglobulins and clinical course

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SUMMARY CSF immunoglobulins were examined in 103 patients with clinically definite multiple sclerosis, 106 patients with either suspected or progressive possible multiple sclerosis and 72 patients with other neurological diseases. Raised CSF IgG index and oligoclonal banding were found in 71% and 75% of clinically definite multiple sclerosis patients respectively and both tests were abnormal in 11% of patients with other neurological diseases. The CSF IgG index and the presence of oligoclonal IgG did not relate to the severity or duration of established disease in these patients. In patients with suspected and progressive possible multiple sclerosis, both a raised IgG index and the presence of oligoclonal banding were found significantly more frequently than in the OND group. Abnormalities of these parameters were significantly correlated with the presence of an abnormal evoked response in these patients ($\chi^2 = 10.16$ $p < 0.01$). When 47 patients with suspected multiple sclerosis were studied prospectively the presence of oligoclonal banding at presentation was associated with development of further disease activity.

Although IgG estimation and electrophoresis in the cerebrospinal fluid (CSF) are well established as aids to the diagnosis of multiple sclerosis, the detection of oligoclonal banding has yielded variable results using differing techniques.¹⁻⁴ Polyacrylamide gel electrophoresis has been reported to be positive in 56% to 94% of patients with clinically definite multiple sclerosis^{5,6} whereas isoelectric focusing with immunofixation has been claimed to be superior with up to 100% abnormalities in clinically definite multiple sclerosis.^{7,8}

More importantly two studies have shown that the detection of oligoclonal banding is of significance for the future development of clinically definite multiple sclerosis in patients with possible multiple sclerosis⁹ and optic neuritis.¹⁰ An association of oligoclonal banding with abnormal evoked responses in patients

with monosymptomatic demyelination has been described.⁹ Patients with benign multiple sclerosis have been reported to have a lower frequency of oligoclonal banding and lower IgG levels in the CSF in comparison to those patients with a more aggressive course.¹¹

In this study we have examined the role of IgG estimation, polyacrylamide gel electrophoresis (PAGE) and isoelectric focusing with immunofixation (IEF) in clinically definite multiple sclerosis and the relationship between CSF IgG abnormalities and disease severity in these patients. We have examined the significance of the CSF IgG index and electrophoresis in progressive possible and suspected multiple sclerosis and the relationship to evoked response abnormalities. In patients with suspected multiple sclerosis the predictive value of oligoclonal banding was evaluated.

Patients and methods

Since 1980 all patients with a clinical diagnosis of multiple sclerosis who were admitted to two Dublin hospitals were surveyed. The multiple sclerosis patients were classified by

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Table 1 Abnormalities of CSF immunoglobulins and evoked responses in various categories of multiple sclerosis and other neurological diseases

	CSF IgG ratio	CSF IgG index	CSF oligoclonal banding			VERs*	BAERs
			PAGE	IEF	Total		
Clinically definite multiple sclerosis N = 103	72/88 (82)†	41/58 (71)	38/55 (69)	39/46 (85)	71/93‡ (75)	81/103 (79)	51/91 (56)
Suspected multiple sclerosis N = 53	22/46 (48)	8/29 (28)	14/33 (42)	9/19 (47)	23/50§ (46)	12/52 (23)	10/45 (22)
Progressive possible multiple sclerosis N = 53	32/42 (76)	16/29 (55)	8/27 (29)	18/20 (90)	26/46 (57)	31/53 (58)	17/45 (38)
Other neurological disease N = 72	30/72 (42)	8/72 (11)	5/42 (12)	3/30 (10)	8/72 (11)	NE	NE

*For abbreviations see text.

†Figures in brackets represent percentages.

‡Eight patients studied by both techniques.

§Two patients studied by both techniques.

|| One patients studied by both techniques.

their attending neurologist according to the criteria of McDonald and Halliday¹² with the modifications that evoked potential findings were not included as evidence of multiple lesions and patients with optic neuritis were included in the suspected category. Patients fulfilling the criteria for three of the diagnostic groups were included in the study: clinically definite multiple sclerosis (103 patients), progressive possible multiple sclerosis (53 patients), suspected multiple sclerosis (53 patients). Patients in the latter two groups are characterised by clinical evidence of a single lesion. For each multiple sclerosis patient, the duration of the illness and disability using Kurtzke's Disability Status Scale¹³ were noted. In addition 72 patients with other neurological disease (OND) had CSF IgG estimations and electrophoresis performed.

Since 1979 we have followed 47 patients with suspected multiple sclerosis and for the purpose of analysis we have included only those followed for a year or longer. Thus although the majority of patients in this prospective study are also included in the main study, patients seen in 1979 are not, while patients with recent onset of symptoms have been excluded from the prospective study.

CSF IgG was expressed as both a ratio to CSF albumin (normal: <0.17) and as the CSF IgG index. The CSF IgG index compares the CSF IgG/albumin ratio in the CSF to that in the serum (CSF IgG × serum albumin/CSF albumin × serum IgG) and was considered abnormal if it exceeded 0.70.¹⁴ CSF albumin and IgG estimations were carried out on a fully automated Behring Laser Nephelometer using Hoechst monospecific antisera. In the initial part of the study CSF electrophoresis using the PAGE technique² was used but this was replaced by agarose IEF with immunofixation⁴ because of difficulties with interpretation of the former when an exudate was present.¹⁵ Serum was examined by IEF in parallel with CSF samples. The CSF and serum electrophoretic patterns were interpreted by one of us (MM) without knowledge of the clinical diagnosis. Visual evoked responses (VERs) and brain stem auditory evoked responses (BAERs) were recorded as previously described.^{15 16} Statistical significance was tested using the chi square and Student's *t* test where appropriate.

Results

Abnormalities of CSF immunoglobulins in clinically definite multiple sclerosis (Table 1)

There were 103 patients in this group. Oligoclonal banding and a raised CSF IgG index was found in 75% and 71% of multiple sclerosis patients respectively while both tests were abnormal in only 11% of the other neurological disease group. Although an abnormal IgG ratio was found in a high proportion of multiple sclerosis patients (82%), raised ratios were also found in 42% of the OND group. Thus, while the CSF IgG index and oligoclonal banding are supportive in the diagnosis of multiple sclerosis, the IgG ratio was of little value.

When patients were divided according to the presence or absence of CSF oligoclonal banding, there was no significant difference in age of onset, duration of illness or disability between the two groups. Similar CSF immunoglobulin abnormalities were found in 23 patients with benign multiple sclerosis (defined as having disability less or equal to three on the Kurtzke Scale after ten years of illness), when

Table 2 Relationship between CSF IgG index, oligoclonal banding and evoked responses in suspected and progressive possible multiple sclerosis

	Suspected and progressive possible multiple sclerosis*	
	with normal ERs	with abnormal ERs
CSF IgG index (Mean ± SD)	0.62 ± 0.32 (N = 29)	1.18 ± 0.81† (N = 29)
Oligoclonal banding	15/47 (32%)	32/47‡ (68%)

*For abbreviations see text

†p < 0.001 (t = 4.07); ‡p < 0.001 (χ² = 12.3)

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compared with nine patients with aggressive disease (defined as having disability greater than three on the Kurtzke Scale within five years of illness). In the benign group the mean CSF IgG index was 1.1 and 19 were oligoclonal banding positive (83%), whereas in the patients with aggressive disease the mean CSF IgG index was 1.0 and eight patients were oligoclonal banding positive (89%).

Abnormalities of CSF immunoglobulins in suspected and progressive possible multiple sclerosis. (Table 1) There were 53 patients in the suspected group and of those examined 28% had a raised CSF IgG index which was significantly more than in the OND group ($p < 0.05$). 46% were oligoclonal banding positive compared with 11% of those with OND ($p < 0.01$). In the progressive possible group the frequency of oligoclonal banding (57%) was similar to the presence of an abnormal IgG index (55%) and both differed significantly from those with OND ($\chi^2 = 10.16$ $p < 0.01$).

All the patients in these two groups (106) had clinical evidence of a single lesion and of these 94 had electrophoresis performed for oligoclonal banding and 58 had an IgG index estimated. Of the 94 who had electrophoresis 47 had abnormal evoked responses suggesting a second subclinical lesion. When these patients were compared with those who had normal evoked responses they had a significantly higher IgG index and a greater frequency of oligoclonal banding (Table 2).

Prospective study of patients with suspected multiple sclerosis

Forty seven patients with suspected multiple sclerosis were followed for a period ranging from one to five years (mean 2.3 years). At initial presentation 26 patients were oligoclonal banding positive and 21 were oligoclonal banding negative. These two groups were similar with respect to age, duration of study period and site of lesion. Fourteen of the oligoclonal banding positive patients (54%) but only two patients in the oligoclonal banding negative group (9.5%) had further attacks during the study

period ($\chi^2 = 10.16$ $p < 0.01$). The remaining 31 patients did not develop features of any other neurological disease and remained in the suspected category for the duration of the study.

The CSF IgG index was estimated in 20 patients in this group. It was abnormal in eleven patients and six of these had a subsequent relapse while of the remaining nine who had a normal CSF IgG index only two suffered a relapse. This difference suggests a predictive value for the CSF IgG index though it is not significant ($\chi^2 = 2.15$, $0.05 < p < 0.1$).

In this group with suspected multiple sclerosis nine patients had evidence of a second lesion detected by an abnormal evoked response (ER) at initial presentation. Four of these patients (44%) developed further relapses during the period of follow up while 12 of the 38 patients with normal ERs (31%) had another relapse. This difference was not statistically significant.

Of the 38 patients with normal evoked responses 17 were oligoclonal banding positive and 21 were oligoclonal banding negative (table 3). The frequency of relapse was significantly greater in the oligoclonal banding positive group ($\chi^2 = 10.56$ $p < 0.01$). Oligoclonal banding was present in a higher percentage of suspected multiple sclerosis patients (55%) than abnormal ERs (19%) and appears to be a stronger predictor of further disease activity. Of the 16 patients who suffered a relapse 14 (87%) were oligoclonal banding positive while only four (25%) had abnormal ERs. This difference was statistically significant ($\chi^2 = 12.6$, $p < 0.01$).

Relationship between CSF IgG index, electrophoresis and diagnostic category of multiple sclerosis

The close association between an abnormal CSF IgG index and oligoclonal banding is shown in the fig. This was significant for the entire patient population ($\chi^2 = 98$ $p < 0.001$) and also for each of the three multiple sclerosis groups. It is clear from the scattergram that the frequency of CSF IgG abnormalities increased in multiple sclerosis as the clinical diagnosis became more certain.

When the two methods of electrophoresis used in this study were compared, IEF with immunofixation detected oligoclonal banding with a higher frequency than PAGE in patients with clinically definite multiple sclerosis (85% v 69%) and in the progressive possible group (90% v 29%). However this difference was only significant in the progressive possible patients ($p < 0.01$) (Table 1).

Discussion

This study confirms the presence of CSF immunoglobulin abnormalities in clinically definite multiple

Table 3 Relationship between relapse rate and oligoclonal banding in 38 patients with suspected multiple sclerosis and normal ERs

	Oligoclonal banding positive	Oligoclonal banding negative
Patients	17	21
Number of patients having a relapse during study period	10	2*

* $\chi^2 = 10.56$; $p < 0.01$

Fig Scattergram of CSF IgG indices of patients with other neurological disease (OND), patients with suspected and progressive possible multiple sclerosis who have normal and abnormal evoked responses (ERs) and patients with clinically definite multiple sclerosis. Closed circles indicate patients who have CSF IgG oligoclonal banding and open circles indicate patients who have no bands. The upper limit of normal for the CSF IgG index is indicated by a broken line (---) and the mean CSF IgG index for each group is shown by a heavy line (—).

sclerosis. Estimation of CSF IgG levels as calculated by the CSF IgG index allowed clear discrimination between the patient group and subjects with OND. The CSF IgG ratio was of little value since a large proportion of OND patients (42%) gave an abnormal result. Similar figures have previously been reported by Link and Tibbling¹⁷ who also felt that this test lacked specificity.

CSF IgG demonstrated an oligoclonal banding pattern in 75% of the clinically definite multiple sclerosis patients and is an important supportive test in making the diagnosis of multiple sclerosis. Two electrophoretic techniques were used to detect oligoclonal banding: PAGE and IEF with

immunofixation. Since these techniques were not compared in parallel but in separate groups of patients no firm conclusions could be reached concerning their relative sensitivities. Nonetheless IEF detected oligoclonal banding in a higher proportion of patients (85% v 69%) and appears to have distinct advantage over PAGE. The main advantage of IEF over PAGE is the superior resolution of proteins which eases interpretation. Because a specific reaction to IgG is examined the presence of haptoglobins and other serum proteins poses no difficulty. On technical grounds IEF is an easier, safer, and less costly method of electrophoresis than PAGE. Concurrent examination of serum allows exclusion of

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samples with identical banding in CSF and serum and the significance of faint bands in the CSF is greater if the serum shows no abnormality. The superiority of IEF has also been claimed in other studies and up to 100% of clinically definite multiple sclerosis patients have been shown to demonstrate oligoclonal banding by this technique.⁸

An abnormal CSF IgG index was found in a similar proportion of clinically definite multiple sclerosis patients as the presence of oligoclonal banding. Since an elevated IgG index is occasionally found in an oligoclonal banding negative patient the index, which gives an objective estimation of CNS IgG synthesis is a useful adjunctive test.

There is evidence that synthesis of CSF IgG increases during disease activity.¹⁸ Nonetheless in this study abnormalities of CSF IgG did not show any relationship with the severity or duration of disease process in multiple sclerosis. Furthermore the presence of oligoclonal banding and an elevation of the CSF IgG index were equally distributed amongst patients with benign and aggressive disease. In contrast, others have reported a reduced frequency of CSF IgG abnormalities in benign multiple sclerosis.^{5 11}

In those patients with suspected or progressive possible multiple sclerosis both a raised CSF IgG index and the presence of oligoclonal banding were found significantly more frequently than in the OND group. In 50% of the single lesion patients an additional subclinical lesion was evident as determined by the presence of an abnormal evoked response (ER). In these patients the finding of an abnormal ER closely correlated with the presence of oligoclonal banding and an elevated CSF IgG index (table 2). This relationship has been previously reported in "possible multiple sclerosis" by Moulin *et al*¹⁹ and also by Bartel and his colleagues.

In the prospective study of patients with suspected multiple sclerosis 54% of those who were oligoclonal banding positive at presentation had further relapses and the majority of these had become clinically definite at follow up. Of the oligoclonal banding negative patients only 9.5% had further relapses. Moulin *et al*⁹ reported similar findings in patients with "possible multiple sclerosis". Twenty four percent of their patients who were oligoclonal banding positive developed further episodes, while only 9% of the oligoclonal banding negative group had a relapse. It has also been shown that in patients with optic neuritis the presence of CSF oligoclonal banding was associated with increased tendency to later dissemination of disease.¹⁰

Patients with suspected multiple sclerosis who had abnormal ERs indicating a second lesion had a higher incidence of further disease activity at follow

up than those with normal ERs. This finding supports the work of Matthews *et al*²⁰ who showed 50% of their patients with suspected multiple sclerosis with abnormal ERs at presentation developed clinically definite multiple sclerosis within three years. However in our prospective study the presence of CSF oligoclonal banding was more informative than that of abnormal ERs, since oligoclonal banding was present in a higher proportion of patients and was a better predictor of further disease activity.

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